

THE DEVELOPMENT OF THE MOUTH AND GILLS IN
BDELLOSTOMA STOUTI

BY

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From the Zoological Laboratory, Columbia University

WITH 36 FIGURES

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Eminent morphologists (Goette, 75, Huxley, 76, and W. K. Parker, 83) have long since suggested the affinities of the Marsipobranchii and the larval amphibia, and Dohrn, 83, has even maintained that the cyclostomes are to be regarded as the descendants of highly organized fishes, possibly teleosts. Beard, 89, furthermore is of the opinion that, "While accepting some degeneration in the Marsipobranchs along with Dohrn, the truth of Balfour's view that they are very primitive forms must also be allowed. They must undoubtedly be added to the gnathostomatous vertebrata. The Marsipobranchii stand between the selacians and ganoids, but far more related to the latter than to the former." The view of Balfour, 85, here referred to held them to be degenerate but not derived from relatively highly organized fish, as is shown by his following words, "Dohrn was the first to bring into prominence the degenerate character of the cyclostomata. I cannot, however, assent to his view that they are descended from a relatively highly-organized type of fish. It appears to me almost certain that they belong to a group of fishes in which a true skeleton of branchial bars had not become developed, the branchial skeleton they possess being simply an extra branchial system, while I see no reason to suppose that a true branchial skeleton has disappeared. If the primitive cyclostomata had not true branchial bars they could not have had jaws, because jaws are essentially developed from the mandibular branchial bar. These considerations which are supported by numerous other features of their anatomy, such as the character of the axial skeleton, the straightness of the intestinal tube, the presence of a subintestinal vein, etc., all tend to prove that these fish are remnants of a primitive and pregnathostomatous group. The few surviving members of the group probably owe their preservation to their parasitic or semiparasitic habits." Whether Balfour's reasoning regard-

ing the jaws and branchial arches can now be upheld will be discussed in the present paper.

Howes, 91, says, "In view of the admitted importance of (the hypothesis in *Petromyzon*) it testifies, to my mind, to an enormity in the gap between the *Marsipobranchii* and the remaining higher *Vertebrata* which even Balfour's conclusion that the former are the remnants of a primitive group, and Haeckel's famous aphorism that they are further removed from the fishes than are the fishes from man insufficiently expresses."

Max Fürbringer, 00, regarding the relationship between the myxinoids and *petromyzontes* states, "In wesentlichen Verhältnissen unterschieden sich die Myxinoiden mehr und principieller von den *Petromyzonten*, als z. B. die *Selachier* von den *Säugethieren*; in einzelnen Merkmalen stellten sie sich selbst weiter ab von den *Petromyzonten*, als diese von den *Gnathostomen* und zeigten zugleich mancherlei Hinneigungen nach den *Akraniern*."

A long list of other workers Dean, 99, Johnston, 05, and Worthington, 05, have recognized in these animals very primitive vertebrates coming as it were between *amphioxus* and the true fishes, and from a study of their embryology and morphology have attempted to arrive at the ancestral or primitive type of vertebrate organs.

Ayers and Jackson, 00, with a few others, are, I think, nearest to the correct position in considering *marsipobranchs* neither as degenerate parasitic forms nor as altogether archaic types, but rather as primitive animals especially adapted to their peculiar life habits.

Recognizing the chaotic condition of opinion regarding these animals Prof. Dean kindly suggested to me that I undertake a careful study of the development of the organs in the head of *Bdellostoma* with the hope that the general problem of myxinoid relationship might be more clearly understood. Von Kupffer had in 1900 published the results of a similar study which he made on scanty and defective material, as is repeatedly mentioned throughout his paper. His study was also confined to rather young stages and, as I have found, stopped really before the most interesting points in the development had been reached.

I am under obligation to Professor Bashford Dean not only for suggesting this study and for his criticism during its progress, but also for his kindness in placing entirely at my disposal his unique series of *Bdellostoma* embryos. I am also glad to express my appreciation of the encouraging interest that Prof. T. H. Morgan has always taken in my work.

GENERAL DISCUSSION OF WORK AND VIEWS RELATING TO THE
MARSIPOBRANCHII.

1. *The morphology of the myxinoids* has been so extensively studied that its literature has become almost cumbersome. The earliest reference to these animals that I have been able to find is that by Gunnerus in 1762. He described *Myxine glutinosa* under the name "Sleep-Marken." He described the tooth plate as a jaw, and also found the two openings, mouth and nose, leading into the pharynx; the remarkable "tongue" muscle-apparatus he called a wind-pipe or air tube. Gunnerus also described the gills of each side calling them lungs. His work is reviewed in the papers of J. Müller, 1835, and P. Fürbringer, 1875.

In 1790 A. J. Retzius also described the tooth plate as a jaw and the club-muscle as a wind-pipe. He classed *Myxine* among the fishes instead of with the worms and molluscs as some previous writers had done.

Abildgaard, 1792, mentioned the "wind-pipe" of Gunnerus as the club-shaped jaw muscle and called the tooth plate, *Zungen-Zähne*, a lower jaw, stating that it was strengthened and moved as such. Abildgaard gave the first correct description of the gills, stating that on each side a canal opened to the outside after receiving the six canals of the gills and, these gills through another set of six canals connected with the throat. He also traced the gill vessels.

Bloch, 1789, described two parts of the club muscle, the outer hollow circular muscle, and the longitudinal one. J. Müller states that he gave their connection incorrectly, claiming both to be fastened to the jaw bone, by which Bloch meant the tooth plate or "tongue;" while in fact Müller claims the first to be attached to the tongue cartilage and the second to the tongue itself.

Home in 1815 described rather fully the gill system and compared the organs of *Bdellostoma* and *Myxine*. Müller states that in his explanation of figures Home correctly said that the teeth belonged to the tongue, he also described the connected muscle-body as a tongue muscle apparatus. P. Fürbringer says that Home first corrected the error of calling the dental-plate the jaw by recognizing in it a tongue.

In the present paper I shall endeavor to show on the other hand that it was Home and subsequent workers up until 1900 that were in error when they called this organ "tongue," and that the earliest workers were correct in interpreting the dental-plate as a jaw.

I have given this brief historical review for the purpose of showing that the idea that the dental-plate is a lower jaw and not a tongue was the oldest or first interpretation of this organ and not a new

idea originating with Ayers and Jackson as one might conclude from reading their papers of 1900. Those authors, it appears, could not have considered these early views in spite of the fact that they are either mentioned or discussed in the papers of J. Müller and P. Fürbringer which Ayers and Jackson have placed in the literature list of their paper.

As Ayers and Jackson state, morphologists followed for a long time Müller in accepting, not his own or original view, but Home's idea that the dental-plate was a tongue. And even Huxley, in attempting to locate the jaw cartilages of *Petromyzon* failed to determine that the tongue was really a part of the jaw structures. Ayers and Jackson in their work on the skeleton of *Bdellostoma* interpreted the cartilages of the tooth plate to be in reality lower jaw cartilages, but this so far as I am able to gather from their paper is all they contribute towards proving the matter. As far as the musculature is concerned they merely note, "The huge club-shaped 'tongue' muscle is made up of the muscles belonging to this arch (meaning the mandibular). These muscles have been in *Bdellostoma* entirely separated from their attachment to the palato-quadrates, and have been translated to their present position in a manner which will be made clear in a subsequent paper," but no description of this process has since appeared.

It had, however, already been shown by Müller and Fürbringer that the muscles manipulating the tongue are innervated by the ramus mandibularis of the trigeminal nerve, *i. e.*, the true lower jaw nerve. Subsequently Allis, 03, in studies on the adult, and Kupffer, 00, (in his figures) on the embryo, have also shown that the dental plate and its muscles are innervated by the true lower jaw nerve. And during the past year Miss Worthington, working under the direction of Dr. Ayers, has traced the cranial nerves and practically repeated Allis' work. She does not, however, refer to this paper and was evidently unaware that her results had been anticipated in spite of the fact that the admirable paper of Allis' had appeared two years earlier.

I do not wish to appear unsympathetic to the work of Ayers and Jackson: indeed, in the following pages I shall join with them in defending the thesis that the so-called "tongue" or dental-plate in myxinoids is really homologous with the vertebrate lower jaw. I must, however, call attention to some of their errors (which I think have in part resulted from their inadequate knowledge of the literature) in my endeavor to place the results of the present paper in a clearer light. Thus they state, for example, that all anatomists "have apparently overlooked the

fact that were this a tongue *or a tongue-like organ* it would be a great anomaly in vertebrate anatomy; since none of the fishes, even the highest fishes, possess a tongue *or even a tongue-like organ.*" It is difficult to understand how the authors could have gained this impression, for one cannot doubt that fishes have tongues and in some cases very perfectly formed ones (cf. Parker and Haswell's Text-book of Zoology, Fig. 807, in which the tongue of the trout is shown and labeled). They would have been correct in stating that among fishes the tongue is not a conspicuous structure and that no fish has a protrusible tongue readily to be compared with the dental-plate of *Bdellostoma*.

Again they mention that "The homology of this organ (referring to the dental-plate) with the vertebrate tongue has never been discussed," while as a matter of fact Neal, 97, three years before, studying the development of the hypoglossus muscles of *Petromyzon*, made the following statement: "I nevertheless consider it highly probable that the so-called tongue of *Petromyzon* is not the homologue of the tongue of higher vertebrates. This conclusion is based on the following grounds: 1. The anterior segment of the *M. parietalis ventralis* remains the same in its relation in *Petromyzon* as in *Ammocoetes*, *i. e.*, without relation to a tongue. 2. While the muscles of the tongue of higher vertebrates are derived from the anterior segment of the *M. parietalis ventralis*, which lies anterior of the hyoid arch, in *Petromyzon* the muscles of the "piston lingual" extend throughout the length of the branchial region and terminate posteriorly in the cartilaginous pericardium. They are quite separate from the *M. parietalis ventralis* which lies lateral and ventral to them. I have stated this evidence of the difference in the relations of the tongue of *Petromyzon* and *Gnathostomata* lest one not familiar with these relations should think that an organ of the same name in these vertebrates should therefore be an homologous organ. As a matter of fact no comparative anatomist has attempted to homologize these two kinds of tongues." This statement refers particularly to *Petromyzon*, but since Ayers and Jackson are discussing the *Marsipobranchii* it seems pertinent in this place. They say that the dental-plate of *Bdellostoma* is homologous with the two pairs of accessory lingual dentigerous cartilages of *Petromyzon*. It appears that they had failed to see Neal's work. On the other hand the work of Howes, 91, is cited in their literature list and indeed quoted on the first page of their paper, yet they have again overlooked or ignored the following remark he makes on pages 134-5: "If, as can hardly be doubted, the 'tongue' of the *Marsipobranchii* is an organ peculiar to them and

unrepresented in the higher vertebrata, the question how far the above-named median ventral cartilage may be comparable to the basi-mandibulo-hyo-branchials of the latter, as Müller, Huxley, and Parker have together sought to show, must remain in abeyance, until more is known of the development of these fishes."

Howes considers the cartilage which Ayers and Jackson have called the cornual to be closely similar to that which Huxley called the Meckel's cartilage in the lamprey and says "with that I hold it to be homologous notwithstanding Parker's view to the contrary." On this point I must disagree with Howes and accept Ayers and Jackson's idea regarding this cartilage, and further with them I entirely agree that the lower jaw structures are to be found in the so-called "tongue" or dental-plate. This position, as I shall endeavor to show in the following pages is admirably supported by embryological study.

2. As far as *Petromyzon* is concerned *the development of the head* has been studied by Kupffer, 94 and 95, Dohrn, 83, and others, although it appears that none of these authors have given particular attention to the peculiar development of the "tongue." Kupffer, 99 and 00, also studied the head of *Bdellostoma* and discussed in detail the development of the mouth, finding as he claims a first or primitive mouth, which is transverse and leads directly back into the gut in a manner similar to the ordinary fish mouth. This primary mouth then becomes closed by a growth of the ectoderm, which Kupffer has called "sekundäre Rachenhaut" the closure persisting until about the time of hatching when the wall is again broken to produce the final or secondary mouth. Max Fürbringer has from this description and for the further reason that the hypophysis always opens into the throat, called the myxincids *Distoma*, the *petromyzontes Cyclostoma*, and the higher vertebrates *Gnathostoma*.

The general embryology of *Bdellostoma* has been beautifully worked out by Dean, 99, but he enters only in a general way into a discussion of the head organs. He gives a very correct account of the gill development from a study of cleared total embryos, the finer details being, of course, impossible to determine in this way. Dean stated that the gills were shifted during development from their original position close after the hyoid arch to their final adult place far back along the sides of the body. He also made the remarkable observation, considering his study to be upon cleared totals, that there is a "doubtful" cleft which is early suppressed lying close behind the hyomandibular, therefore corresponding to the "thyroidean" cleft of Dohrn. Dean also, very cor-

rectly, I think, failed to find any correspondence between branchiomery and myomery.

Price, 96, was the first to describe embryos of the myxinoids. He studied three stages in the development of *Bdellostoma* and in the youngest embryos found a narrow canal extending from the anterior end of the mouth cavity into the cavity common to the nose and hypophysis, while in the older stages this canal was closed. M. Fürbringer saw in this observation very strong evidence in favor of Dohrn's idea that the primitive mouth of vertebrates is represented by the hypophysis.

Price, working with a limited supply of material made the mistake of supposing that a great number of gill pouches appear during development, possibly as many as thirty-five. Of these, he thought the posterior ten or fourteen develop into the gills of the adult while the others entirely disappear. Dean corrected this statement when he found a shifting and not a closing of the gills to take place; and Price himself, in a subsequent paper on the development of the excretory organs of *Bdellostoma*, based on a more complete series of embryos, has corrected his former statement and accepted Dean's interpretation on this point. Notwithstanding these corrections Price's original idea has since been utilized by Ayers and Jackson, 00, who try to account for the atrophy of the forward gills as due to the enormous development of the "club muscle" in that area. Johnston, 05, also quotes Price's earlier conclusion and states his belief that there is a reduction of the anterior gills on account of the parasitic life of this animal. It has been clearly shown, however, by Howes, 91, Ayers, 93, Jordan and Everman, 96, and Worthington, 05, that these animals are not parasitic but predaceous, attacking disabled fishes. Therefore Johnston's explanation will not suffice. Since, also, there are no forward gills lost we must accept the position that the gills belonged originally to the head region, and owe their extension into the trunk to the shifting forward of trunk myotomes into the occipital region, the view, by the way, which Johnston rejected in favor of his explanation cited above. All of these various disagreements concerning the development of the myxinoids have come within the last few years; Price's initial work on the subject appearing in 1896. It may be mentioned that only a few workers, about half a dozen in all, have been so fortunate as to obtain the material.

This review of the various and contradictory opinions might easily be extended, for no author has seemed to consider his paper complete without taking a new position regarding either the significance of this group of *Marsipobranchii* or the importance of the evidence furnished

by some organ that he may have studied. Enough, however, has been said to show the need of further embryological observations.

METHOD AND MATERIAL.

The material upon which the present results are based was collected in the Bay of Monterey, California, mainly during the summers of 1896 and 1899 by Professor Bashford Dean. This constitutes the most complete series of *Bdellostoma* embryos that has been procured. The younger stages were fixed for the most part in Graf's picro-formalin, the later embryos being killed, after removal from the parchment-like egg-shell, in sublimate-acetic. In most cases these fixatives have given very satisfactory results.

The sections were prepared by Prof. Dean, Dr. L. Neumeyer, of Munich, and Dr. N. Yatsu, formerly of this laboratory. The embryos are very difficult to prepare, but with such an abundance of sectioned material I have been able to select a complete and satisfactory series, and I am confident that none of my observations are open to the criticism that they have been made upon defective material. The stains used were Heidenhain's iron hæmatoxylin, Delafield's hæmatoxylin, alum and borax carmine, all of which give good results.

THE DEVELOPMENT OF THE MANDIBULAR ARCH.

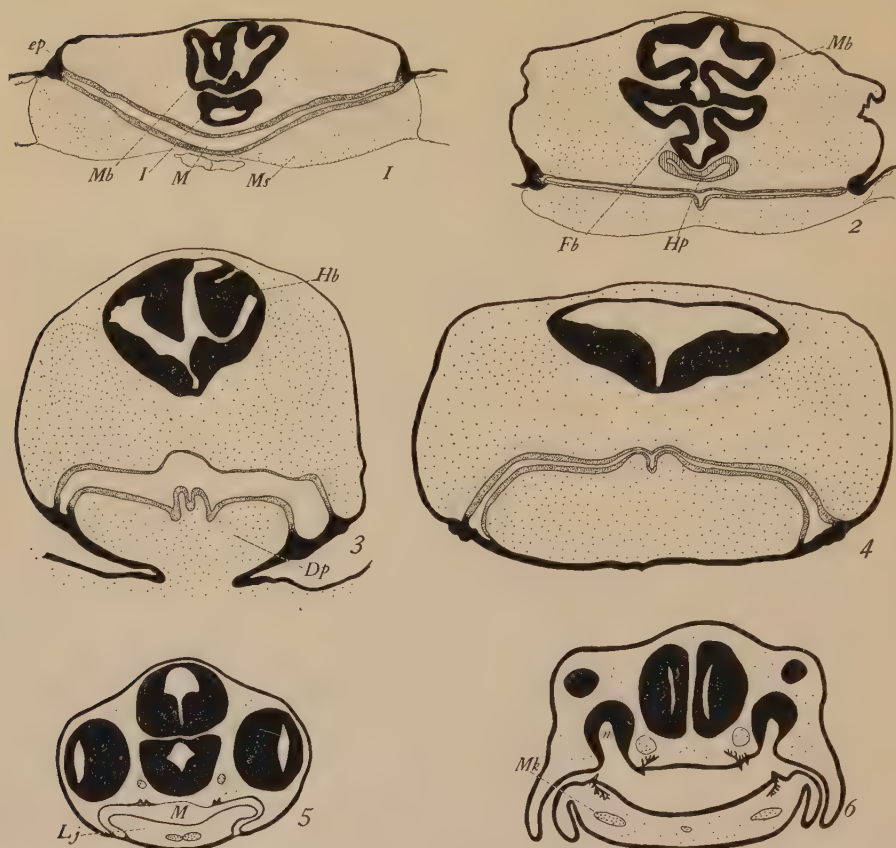
In the young of *Bdellostoma* one finds, to my mind, a most striking confirmation of Dohrn's view that the mouth of vertebrates is a modified gill arch. In these embryos the mandibular cleft passes through a series of changes during its development which are most suggestive in the light of Dohrn's thesis. Very young embryos, such, for example, in which the nose is still a single tube leading directly into the mouth cavity and in which six or seven gill slits are present on the laterally outspread plates (as illustrated in Dean's Fig. 37, pl. 18, and Fig. 101, pl. 22), will show the mandibular cleft in the following condition: Throughout the greater portion of its extent it is strongly arched with its lateral diverticula directed in an oblique dorsal direction and coming in intimate contact or actually fusing with the ectoderm. This is so strikingly analogous to the way in which a gill cleft meets the ectoderm that no observer could fail to be impressed with the similarity. Fig. 1, a cross section through the widest portion of the mandibular cleft of an embryo at this stage, shows the cleft fusing laterally with the ectoderm and having an arch or curve resembling that of all the following gills. The hyomandibular of the same embryo may be seen for comparison in

Fig. 15. The stippled area of these figures does not necessarily indicate endoderm but merely the epithelial lining of the mouth and pharyngeal cavities. In some figures this, of course, is actually ectoderm. The outer body wall and brain sections are indicated in black.

An embryo slightly older than the former, having almost lost its nasal communication with the throat and having one or two more gill clefts present, shows the mandibular cleft as follows: The arch of the younger embryo has almost entirely disappeared, the ventral mesenchymatous tissue, Ms, has thickened below the throat cavity and seems to have pushed the middle curve of the arch up until in section it appears as a horizontal cleft extending from side to side of the head. In this condition the intimate fusion of the diverticula with the ectoderm still persists. A slight median furrow now runs along the floor of the throat throughout the region of the mandibular cleft. Fig. 2, a section through the widest portion of the mandibular region, illustrates these changes. Somewhat further caudad this arch also has its lateral borders directed slightly dorsal, as a reminiscence merely of the condition of the younger stage. The hyomandibular and other gill clefts of this embryo differ but slightly from those of the younger one.

A somewhat more advanced embryo, measuring about 15 mm. in length, shows the mandibular cleft with its lateral portions curving down ventrally although still fusing with the ectoderm. Fig. 3 shows this condition in cross section; the embryo was slightly shrunk so that the mouth cavity is exaggerated in the figure, yet the increase in the ventral mesenchyme and all points are well shown. The hypophysis, which is seen in Fig. 2 has terminated before reaching this corresponding region in the older embryo. The anterior part of the head has become much longer, a great nose region now extending in front of the forward end of the gut. This section is also far posterior of both the eyes and of the infundibulum while a similar part of the mandibular cleft in Fig. 2 was only one section posterior of the eyes and the infundibulum had not been reached, the fore-brain being shown bent under the mid-brain.

Fig. 4 is a section through the mandibular cleft of an older embryo. In this stage all of the gills have appeared, but are still spread out on the lateral plates, and the nose exists at this time as two parallel tubes, a condition occurring also in the embryo of Fig. 3. The present section shows clearly the ventral inclination of the lateral diverticula of this cleft, as well as the ectoderm pockets, which appear almost ready to break through and form the lateral mandibular gill openings, a process, however, which does not take place: it is only at a later period that the definite



FIGS. 1 to 4 illustrating the development of the mandibular arch in *Bdellostoma* $\times 40$ diameters, with Figs. 5 and 6 of this arch in *Triton* for comparison. Fig. 1. Section through the widest part of the mandibular arch in a very young embryo. *ep*, ectodermal mandibular "gill" plates; *I*, infundibulum; *M*, mandibular cleft; *Mb*, mid-brain; *Ms*, ventral mesenchyme. Fig. 2. Widest portion of the arch in slightly older embryo. *Fb*, fore-brain; *Hp*, hypophysis. Fig. 3. Section of older embryo showing ventral turn of the mandibular diverticula. *Hb*, hind-brain; *Dp*, dental-plate anlage. Fig. 4. Section of a still older embryo showing the wide mandibular cleft in actual contact with the ectodermal body wall. Fig. 5. Section through the mandibular arch of *Triton*, after Greil, compare with Figs. 3 and 4. *Lj*, lower jaw anlage; *M*, mandibular cleft. Fig. 6. An older *Triton* embryo after Greil, compare Fig. 11 after considering text. *Mk*, Meckel's cartilage; *n*, internal nares.

mouth opening is formed. Here, however, it is seen that the entire portion or mass of embryonic tissue below the mandibular cleft has increased considerably in bulk and that the trough-like furrow still extends along the mouth floor in this region. The furrow deepens during development and finally cuts or separates the ventral tissue into two forward halves, to be described in detail further on. All of this ventral mass is the anlage of the so-called "tongue," it is seen to be enormous and much too far forward for the anlage of such an organ. In all respects, however, it corresponds in position and extent to sections of the lower jaw portions of most embryos.

Two of Greil's, 05, figures which appeared in his paper on the development of the mouth in Triton are shown in outline, Figs. 5 and 6, to illustrate the identity of the ventral tissue in my figures with the lower jaw tissues. From this identical ventral tissue, as will later be seen, the true lower jaw or so-called "tongue" develops in a remarkable way. Fig. 10 shows the mandibular arch greatly curved, but if the embryo at this age was flattened dorso-ventrally, as it is at the stage from which Fig. 4 was made, then Fig. 10 would give the curve of the mandibular arch similar to that seen in Fig. 4.

THE DEVELOPMENT OF THE "TONGUE" SHOWS IT TO BE THE HOMOLOGUE OF THE VERTEBRATE LOWER JAW.

It may be said that almost nothing is known of the "tongue" development in myxinoids. This is strange, since one would think that an organ so unique and peculiar as this one is would have been examined by the first workers that procured the embryos of these animals. I wonder particularly that Kupffer in studying the head development in *Bdellostoma* did not center his attention upon this organ instead of giving it only casual mention.

Göppert, 02, made in substance the following statement in Hertwig's handbook of embryology, L. 6, p. 36: "The tongue of *Petromyzon* is first attained when the ammocoete metamorphoses, as the complete change of life is contemporary with the transformation of the structures. The cartilage of the tongue arises according to P. Bujor, 91, in the massive connective tissue of the ventral wall of the larval mouth. The same author found the tongue muscles evidently first arising from the floor of the mouth in the neighborhood of the velum and thyroid gland. Bujor says that they have nothing to do with the tongue muscles of the higher gnathostomes; and they are controlled exclusively by the vagus according to Neal, 97, who finds that the so-called *ramus recurrens*

vagi is homologous with the hypoglossus nerve of the higher vertebrates." As cited above in the general discussion Neal has shown that the anterior segment of the *M. parietalis ventralis* is without relation to the tongue in either *Ammocoetes* or *Petromyzon*, while the muscles of the tongue of higher vertebrates are derived from this segment. I am sorry not to be able to give at first hand any statement regarding the so-called "tongue" of *Petromyzon*, but from a study of the literature it seems that we are dealing here with an organ possibly as untongue-like as is the dental-plate of *Bdellostoma*. Göppert says regarding the myxinoids, that since they fail to have a larval form the tongue is found arising here much earlier than in the petromyzontes. At the termination of embryonic development the tongue has attained its final form and functions from then on as the boring apparatus. He states that Kupffer, 99 and oo, described and figured the massive anlage of the tongue in *Bdellostoma* as arising from the mesodermal tissue in a stage when the "secondäre Rachenhaut" still existed. Göppert concludes with the pertinent remark that the cyclostome tongue remains a peculiar structure in contrast to the more simple tongue of the gnathostome fishes.

Dean, 99, p. 269, refers but briefly to the tongue, stating merely, that below the opening of the hypophysis into the gut the tongue arises on the ventral wall of the throat as a paired outgrowth, and its rapid development greatly modifies the shape of the mouth cavity.

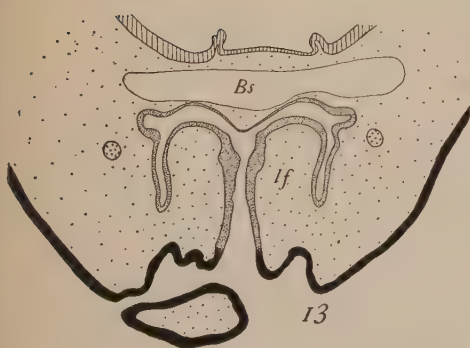
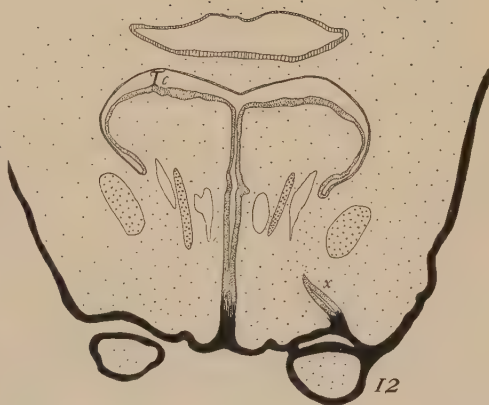
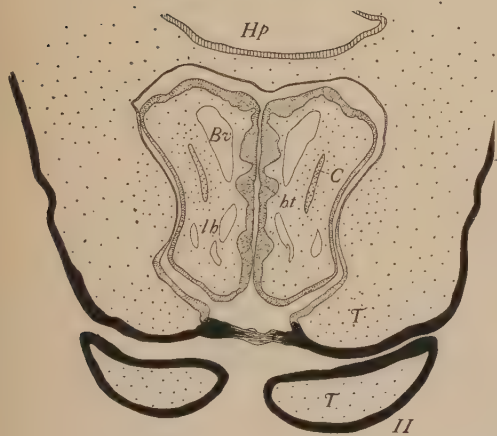
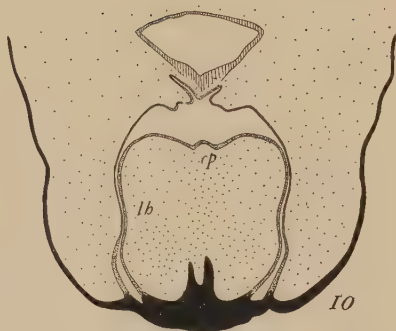
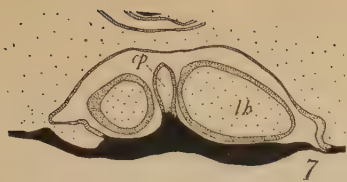
According to my more detailed studies the stages in the growth of the tongue are as follows: The earliest anlage of the dental-plate, as I shall term it from now on, is seen in Figs. 3 and 4 to be represented merely by the thickening of the mesodermal tissue in the ventral mouth region. This tissue composes the entire body of the embryonic head below the throat floor just as the lower jaw tissue does in embryos of *Triton*, Figs. 5 and 6, *Cestracion*, and other lower vertebrates. This mesodermal mass continues to increase in size and to become thicker in a dorso-ventral direction, while at the same time its anterior end becomes free from the lower body wall and divides into two distinct prongs or forward extensions, Fig. 7 lh. It will be seen that these prongs a short way back from their anterior tips come together in a thin vertical piece, Fig. 7 cp, which now alone connects them with the ventral wall of the head. It may be remarked that Fig. 7 is taken from an embryo in which the first four branchial gills have been drawn in to the head region. In Fig. 8, which is a section through the same embryo taken further tailward, we see the two lateral horns, lh, joined to the median portion, cp, and on the right side of the figure, the section being slightly oblique, the lateral prong has also joined the ventral body wall. Here

again the mandibular cleft turns ventro-laterally; and, as before, the entire tissue below the cavity of the throat is the dental-plate anlage corresponding in position and extent to the lower jaw tissues of all vertebrate embryos. From these figures one might suggest that the median body may still represent a tongue, but it will at once be seen that the sections pass through the region of the hypophysis and are thus anterior ones. They are obviously too far in front of the hyoid cleft to represent the tongue anlage. I have looked with the greatest interest hoping to find some indication of a tongue in these embryos, as I would then be assured that the other apparatus was certainly not such an organ. My failure to find it, however, is not surprising in view of the fact that the tongue is inconspicuous in most fishes, and may even be entirely wanting in some. It may not be out of place to recall here that the fish's tongue is essentially a projection of the forward part of the hyobranchial apparatus with the mucus membrane of the mouth covering the thickened submucosa. Its development is, therefore, intimately connected with that of the skeleton.

Fig. 9 shows the condition of the dental-plate as illustrated by a section passing through the anterior part of the organ. In this embryo the gills are completely drawn into their position on the sides of the neck, *i. e.*, not spread out laterally as in the previous stages. They have shifted a considerable distance back of their original position, so that a long interval now exists between the hyomandibular cleft and the first branchial gill. In none of the embryos mentioned above had any shifting of the gills taken place. The gill pouches it may here be noted are just beginning to be formed, a process which will be described further on. This embryo is also interesting as being the youngest one in which the anlage of the thyroid gland makes its appearance, Stockard, 06. The anterior ends of the fore-gut and of the nasal canal are directed ventrally and they are now more caudad in position than when fully developed.

The two lateral prongs of the dental-plate anlage have grown further forward than the median copular portion and are seen to have become thicker dorso-ventrally, as has, also, the entire head of the embryo. For this reason the mandibular cleft has now a strong curve, but still comes in intimate contact with the ventral body wall, being prevented from opening to the outside only by the sekundäre Rachenhaut of Kupffer. Two median tentacles are seen below in cross section showing their cores of mesoderm stippled. As we pass caudally in this same embryo to a place shortly anterior of where the Nasenrachengang, Hp, opens into the throat we find such a section as is seen in Fig. 10. This shows

FIGS. 7 to 14. Sections showing the development of the dental-plate or lower jaw $\times 41$ diameters. Fig. 7. Through the anterior part of the mouth showing the dental-plate anlage in a young embryo. *cp*, the thin median body; *lh*, two large anterior prongs, all of these parts are united further back. Fig. 8. A more posterior section of the same embryo, the median body, *cp*, is here united with the anterior prongs, *lh*. The anterior prong on the right side is seen united also with the ventral wall of the head. *M*, mouth cavity. Fig. 9. Section of the anterior portion of the dental-plate in older embryo, *lh*, the anterior prongs have grown ahead of the median body in their forward extension. *Hp*, hypophysis; *T*, tentacles. Fig. 10. Section through the same embryo in a more posterior region than Fig. 9. The anterior prongs, *lh*, are here joined to the median body, *cp*. The entire dental-plate anlage has its ventral wall in common with the ventral wall of the head just as has the lower jaw of the Triton embryo in Fig. 5. Fig. 11. Section through the anterior prongs, *lh*, of the dental-plate showing the teeth anlage on their median faces. *ht*, teeth; *Bv*, blood-vessels; *C*, mandibular cartilage; *T*, section of tentacle; *T'*, section through base of more posterior tentacle. Fig. 12. A more posterior section than Fig. 11, the two forward prongs of the dental-plate are joined together medio-ventrally, and laterally to the head. Their ventral wall is the ventral wall of the head as would be expected of the lower jaw. *Tc*, throat cavity; *x*, a place where the forward prong is still free from the head not far enough posterior to give the complete union. Fig. 13. Through the longitudinal mouth of an old embryo. *lf*, fleshy lip folds the anterior lateral extensions of the dental-plate body; *Bs*, blood sinus. Fig. 14. A more posterior section through the same embryo. Shows dental-plate with teeth, *ht*, developing on its dorsal surface; *C*, basal cartilage of dental-plate; *Tc*, throat.



that the two forward lateral prongs have, as we traced them, posteriorly fused with the middle copular portion, which is shown in the figure as the median ridge on the throat floor and as a ventral triangular protrusion of the median mesoderm. The lateral prongs have also become joined to the ventral wall of the head. Four sections back of this one they join the head laterally also, and thus the vertical side spaces seen in the figure are lost, leaving only the upper space as the throat cavity. It is plainly seen that the entire mass of tissue ventral of the throat cavity and included in the forward mandibular arch is the tissue that should form a lower jaw in an embryo, and in no particular the tissue to form simply the tongue of an animal. Its extreme anterior origin is in itself strongly against such a view.

Development continues without any marked points of interest until we examine an embryo of about 28 mm. in length. This may be described as follows: The mouth opening is almost formed, as the secondäre Rachenhaut is beginning to disintegrate, the gills have shifted backward, being more than twice as far posterior of the auditory vesicles than these are from the anterior point of the head. The gills are well pouched and the cartilage arches or cuffs are forming about their external tubes. A large and well-formed club muscle extends from the velar region to the first gill, this organ only appears after the gills have shifted some distance back of the hyoid cleft. The circular muscle of the club first arises as two muscle bodies some distance apart and dorso-lateral to the longitudinal one, and as it develops the circular muscle migrates ventrally and surrounds the long one.

This embryo has its dental-plate extending forward as two large prongs which may be traced a long way back before the median copular portion is reached. The prongs are thus seen to grow ahead of this piece in their forward development, for we recall how close to their anterior ends the copular portion was found in the embryo of Figs. 7 and 8. The early anlage of the teeth now appear on the median faces of these two prongs. The teeth are here entirely derived from the epiblastic wall. Cartilages are developing one to each prong of the dental-plate and its substance is very vascular, enclosing large blood sinuses. Fig. 11 is a section through the anterior region of the dental-plate and shows two tentacles, T, cross sectioned below, while two others, T', are cut through their base of attachment. The Rachenhaut is disintegrating along its middle portion. The cartilages of the two prongs are shown coarsely stippled and the numerous blood vessels, Bv, are indicated in outline, two enormous sinuses being located one along each dorso-median border of the prongs. The teeth anlage are represented by

the thickened portions of the epiblast. Tracing the organs caudally through the series we find the two dental-plate bodies becoming joined to the head laterally and joining one another medio-ventrally until we reach a section, Fig. 12, where the lower surface of these dental-plate bodies forms the ventral wall of the head. They are thus in all respects similar to a lower jaw in position and composition, and have as far as I am able to interpret nothing about them suggestive of a tongue. The median furrow left by the incomplete dorsal union of the two prongs runs for many sections through the throat region, then is lost as the thin middle body referred to above comes between the two halves of the throat floor and forms a slight ridge along the median line. The hypophysis, seen in Figs. 11 and 12, runs back for quite a distance before opening into the gut.

As development progresses the two anterior parts of the dental-plate become attached laterally to the head further and further forward until they cease to project as free portions. Studying an embryo 43 mm. long, in which the brain has flattened and become almost solid as in the adult, and in which the nasal opening is now anterior and terminal, instead of ventral as in younger stages, we find that the mouth is a longitudinal opening much as it is in the mature condition. This opening or longitudinal mouth is easily comparable with the ordinary transverse mouth of other vertebrates when we remember that in the myxinooids the forward portions of the lower jaw, which are hardly more than lip folds, have remained separate in the old embryos and that the tooth bearing portion has been carried to the back of the mouth. Now if we should unite medianly the forward projections of the dental-plate, the mouth could only open as an anterior-ventral transverse slit, or a slit in front of the lower jaw border as it does in other vertebrates. Referring to Dean's Fig. 113 of a total embryo, the anterior prongs of the dental-plate are indicated by shaded portions, and the lighter median streak shows their forward separation. The figure makes clear the close similarity to a transverse mouth. Dean writes, p. 263, that, "The mouth cavity suggests closely that of a gnathostome; in ventral aspect it appears as a narrow crescent whose convexity is directed forward."

The longitudinal condition of the mouth will be made clearer by examining sections of the 43 mm. embryo. Fig. 13 is through the mouth opening and the two forward lip-like folds of the dental-plate, If, are fused laterally with the head. One of the tentacles, T, is sectioned below. As the two lip-like bodies are followed back through the series they are found to be continuous with the dental-plate of which

they are in fact the forward fleshy extensions. Fig. 14 is a section further back in which the two forward parts have fused medianly to form the dental-plate, and on this bilateral body the teeth are developing. These now appear upon its dorsal surface instead of on the two median surfaces as in Fig. 11, for the median surfaces which faced one another when the teeth began to form, now spread apart and become the final upper or dorsal surface of the dental-plate. The thin cartilage of the plate is seen below the dental surface.

From the foregoing account a critic might object that, if we interpret the tongue as the lower jaw, we have a jaw of a most unorthodox type, for it develops in such a way that one portion of it may be moved out between its two other parts; a condition which seems at first sight mechanically impossible. But it must be remembered that the forward attached parts are only the fleshy folds of the jaw, mere lip-like structures, while the dental-plate itself has a sheet-like membrane spreading out from its four sides and loosely folded about its edges. This membrane is continuous with the lining membrane of the mouth cavity. By means of the highly modified muscle system the tooth-plate may be lifted up and carried forward, owing to its loose-folded membranous connection. The movements, moreover, in the living animal have recently been explained by Worthington, 05.

It follows finally from the discussion of the development of the mandibular arch and dental-plate in *Bdellostoma* that the organ long called the tongue in myxinoids is in no sense comparable or homologous with the vertebrate tongue. In this case, therefore, it should no longer bear so misleading a name, since the word tongue must necessarily imply or suggest a tongue-like structure. The term dental-plate which has been applied by several writers to this organ seems a very appropriate one. It indicates the nature of the organ and in no way interferes with the understanding that it is actually homologous with, though superficially widely different from, the lower jaw of all other gnathostomes. The Marsipobranchii have been so repeatedly placed among the Gnathostomata that the group will scarcely seem new in its correct position.

THE DEVELOPMENT AND FATE OF THE HYOMANDIBULAR GILL CLEFT.

This gill cleft in the youngest stages is strikingly similar to the mandibular, both having extensively developed diverticula bending upward in a manner typical for all of the gills. Dean has stated, p. 270, that the mouth is distinctly paired in character, but unlike the hyomandibular pouches, its diverticula are shown in section to bend downward,

instead of upward, and to fuse at their margins with the ectoderm, thus suggesting the gill slit mode of origin of the mouth so elaborately defended by Dohrn. This statement is correct except for the very young stages in which, as I have shown, the mouth diverticula also bend upward, Fig. 1, and suggest, therefore, much more forcibly the gill slit origin of this organ.

The hyomandibular pouch follows close after the mandibular, which in all stages is the most anterior, and there are no premandibular diverticula of the gut, which Dean indicated from his study of surface preparations.

The very young embryo described as having the nose tube leading directly into the gut, and shown in Dean's Figs. 37 and 101, presents the hyomandibular pouch in the following condition. It is situated well in front of the auditory vesicles and is enormous in extent, as is shown in Fig. 15, a section of the same embryo from which Fig. 1, of the mandibular arch, was taken. In this section it will be seen that well-marked ectodermal pockets are present and a corresponding thickening of the endodermal gut wall is in close contact with them. It thus appears as if this gill is about to establish an opening to the exterior, but such is not the case since from this stage onward there sets in a steady retrogressive development.

Fig. 16 shows a section through an embryo in which the mandibular arch has flattened out or become horizontal, no longer curving upward, the condition shown in Fig. 2. Fig. 16 is through that part of the hyomandibular pouch where it comes in closest approximation to the corresponding ectoderm pocket. The gut pouch, therefore, is decreasing in its lateral extent, while in cross section the lateral diverticula become retort-shaped, large bulb-like chambers forming their extremities. The endodermal thickenings in the present stage are not so marked as in Fig. 15. As the embryo continues to develop the ectodermal plates or thickenings become gradually less pronounced until in embryos of about 15 mm. in length there appears no ectodermal evidence of the hyomandibular, Fig. 17. The bulb-shaped extremities of the gut diverticula in Fig. 17 are more definitely marked off than in the younger stage of Fig. 16, here, also, the hyomandibular cleft is behind the auditory vesicles instead of being anterior to them as in the younger embryos. In the matter of position the relation of the hyomandibular to the auditory vesicles is slightly variable in embryos of the same developmental stage, but as development progresses the ear-vesicles always attain a more anterior position. This fact is probably due to the forward shortening or

condensation of the brain, though I shall be better prepared to discuss this subject after a more careful study of the brain development.

The hyomandibular cavity continues to degenerate and there is only a slight indication of it in embryos of later stages, *i. e.*, those in which the branchial pouches and head cartilages are forming. In Fig. 18 through the posterior auditory region of such an embryo the hyomandibular is indicated by the slight lateral diverticulum marked Hm just at the beginning of the throat where the gut wall arches dorsally to map out the velum. Older stages lose even this indication and the gut arches up around the anterior end of the velum without any suggestion of the hyomandibular diverticula.

Thus in *Bdellostoma* the hyomandibular cleft early attains an enormous development, then gradually decreases in size, and disappears before the embryo has reached a very advanced stage.

THE PRESENCE AND FATE OF TWO PAIRS OF POST-HYOMANDIBULAR GILL CLEFTS.

Dean, 99, writes (*op. cit.*, page 270) that the question of the disappearance of a pair of gill clefts lying post-hyomandibular has been strikingly revived by his studies. In a foot-note on the same page he also mentions this "doubtful cleft" which lies close behind the hyomandibular as corresponding to the thyroidian cleft of Dohrn. Studying sections of these embryos I have been able to confirm the observations made on cleared totals by Dean and I have further demonstrated a second cleft following close behind the first, which likewise disappears during development.

The existence of these additional clefts in the embryo is of evident interest with regard to the maximum number of gill slits in chordates. Since the adult *Bdellostoma* often possesses as many as thirteen pairs of gills we may now include from embryonic data a total number of fifteen pairs exclusive of the hyomandibular and mandibular clefts. There seems to be no valid argument against this greater number representing a primitive feature. The tendency in *Bdellostoma* is to lose its large number of gills as is shown first by the suppression of these two embryonic pairs and further by the observations of Miss Worthington, 05, on the adult. She has found (p. 633) the number of gills in different species ranging from seven to thirteen pairs, and in the case of the Californian species no number seems to be either normal or fixed. Of more immediate interest is her observation that traces of lost gills are often to be found not at the hinder end of the series of gills, where one would at first suppose, but at the cephalic end.

The two pairs of post-hyomandibular or thyroidean gills, (neither name, unfortunately, designates them accurately) always remain in their original position, not being affected by the shifting process which carries the true branchial gills back into the trunk region. In this connection it is important to note that these two gills are always drawn well into the head region and never occur out on the lateral gill plates where all of the following ones first make their appearance. The thyroidean gills disappear soon after the shifting starts.

The young embryos in which the diverticula of the mandibular arch bend upward show these gills in the following condition. In Fig. 19, which passes through the first of these two gills, the ectoderm forms a depressed pocket and is much thickened where the gut diverticulum comes close to it, the endoderm also thickening. This is in the anterior auditory region. The second thyroidean gill is much the same and is found in the posterior auditory region, one thus following close after the other. The next gill, which is destined to form the first true branchial, follows at an equal distance behind the second and may be seen for comparison in Fig. 23. This is not so well-developed as the thyroidean gills, being further caudad in the series and the developmental stages are gradually less advanced as we pass tailward.

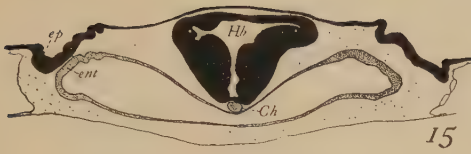
In embryos which have the mandibular arch flattened horizontally and which show seven or eight gill clefts, the first thyroidean gill as shown in Fig. 20 has a well-marked ectodermal pocket and the corresponding gut diverticula is greater in extent than that of any following gill. Fig. 24 shows the first branchial gill in the same embryo. It is situated just at the base of the laterally outspread gill plate. The first thyroidean gill is again in the anterior auditory region and the second stops just as the auditory vesicle fades out posteriorly. In Fig. 20 the slight nick, 2t, just ventral of the first thyroidean is the most anterior tip of the gut diverticulum for the second thyroidean; all of the gills having an obliquely caudal direction from pharynx to body wall.

When the embryos are 15 mm. long (a stage in which the mandibular arch is as seen in Fig. 3 and the hyomandibular in Fig. 17) the first thyroidean gill is in the condition shown in Fig. 21. It is still in the anterior auditory region, but one will note that the ectodermal thickenings and pockets have now disappeared as have the hyomandibular ectodermal thickenings at this stage, Fig. 17. The gut diverticulum will be noticed now to extend little more than one-half of the distance from the gut to the ectodermal wall. The second thyroidean gill presents much the same appearance, the two are therefore undoubtedly degenerating

FIGS. 15 to 18 illustrate the degeneration of the hyomandibular cleft $\times 44$ diameters. Fig. 15. Through the widest portion of the cleft in a very young embryo. On the left side the endodermal and ectodermal thickenings are almost in contact. *ep*, ectodermal hyomandibular gill pocket; *ent*, endodermal thickening; *Ch*, chorda; *Hb*, hind-brain. Fig. 16. Section of an older embryo showing increased space between ectodermal and endodermal parts of the gill. Fig. 17. Section of still older one. The ectodermal thickenings have disappeared and the endodermal diverticulum, *Hm*, is less extensive; *v*, anlage of velum. Fig. 18. Section of old embryo, the slight diverticulum, *Hm*, is all that remains of the hyomandibular cleft. *A*, ear; *Ca*, cartilage of the auditory box.

FIGS. 19 to 22. Sections showing the degeneration of the first post-hyomandibular gill. Lettered as above. Fig. 19. Section of a very young embryo. Fig. 20. Older embryo showing the gill further developed. *2t*, the anterior beginning of the second post-hyomandibular gill. Fig. 21. Section of older embryo showing degeneration of the ectodermal thickening, and the endodermal diverticulum is less extensive at this age. *p*, pharynx. Fig. 22. Embryo in which this gill is almost lost, there being no ectodermal indication of it, and the throat diverticulum is very small.

FIGS. 23 to 25 show sections of the first branchial gill in early stages of its development. Fig. 23. From the same young embryo as Fig. 19. Fig. 24. From the same embryo as Fig. 20; *np*, branchial nerve placode. Fig. 25. Older embryo with the endodermal diverticulum, *ent*, much increased in extent; *g2*, *g3*, anterior edges of the diverticula of the 2d and 3d branchial gills. Fig. 26. Guide to indicate the embryonic areas from which the above sections were taken. *Hm*, region of the hyomandibular ones. *T*, the first post-hyomandibular ones. *Br*, the first branchial gill region.



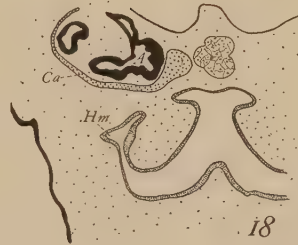
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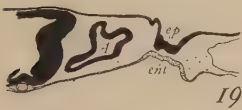
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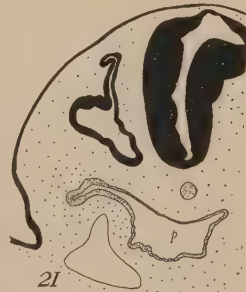
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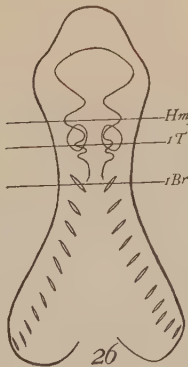
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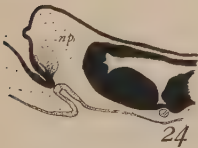
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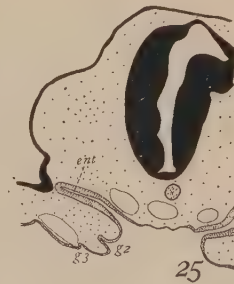
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while the first branchial, seen in Fig. 25, has progressed considerably beyond the condition shown in Fig. 24.

Fig. 22 shows in an older embryo a stage of still further degeneration of the first thyroidean gill. Here again there is no ectodermal indication of the gill, and the gut diverticulum extends somewhat more than a third of the distance from the pharynx to the body wall. Finally in older embryos these gut diverticula become less and less important until they are lost entirely. Thus they cannot be found in a stage when the gills are beginning to form the pouches and have shifted back into the trunk region.

Including the mandibular I have thus far described the developmental changes taking place in the four anterior pairs of gill clefts. The development of the mouth arch is progressive and highly modified; beginning as an ordinary gill cleft extending dorsally, it changes the position of its diverticula into a more and more ventral position, and finally undergoes complicated changes in connection with the development of the dental-plate.

The three following gills, hyomandibular and the two thyroidean, undergo a distinct retrogressive development: in the young embryo they are the most advanced elements of the entire gill series; they then gradually degenerate and in late stages they become entirely lost. On the other hand, the first branchial cleft as we have seen develops progressively, and becomes the first true marsipobranch of the adult. All of the following gills progress in a similar manner and reach the mature condition in a way to be described below.

THE DEVELOPMENT AND SHIFTING OF THE TRUE BRANCHIAL GILLS— POUCHES, ARCHES, ETC.

The development and changes in the relative position of the gills in *Bdellostoma* are among the most interesting phases in the embryology of a Myxinoid. Dean in his study of cleared embryos described the general processes of the gill development with remarkable correctness, so that one studying the finer details in serial sections is surprised at the manner in which the general points of development were interpreted from the total embryos.

On page 261 Dean says, "In the hinder (head) region we have thus a view of the mode of closure of the fore-gut: and we note the drawing together of the sides of the gill-lappets and the mode of backward extension of the ventral rim of the definitive pharynx, in a way which suggests curiously an analogy with reptiles and birds." This description is just

such as one would give after a study of sections and when the large yolk-bearing egg of *Bdellostoma* is recalled, resembling so closely the eggs of reptiles and birds, we would expect the mechanics of development to operate in a comparable fashion, *e. g.*, the closing together of the gut walls. We find on page 264 in a discussion of late embryos, that a great increase in the length of the neck region has taken place, the head now advancing around the anterior end of the egg. "With this continued growth the further change in the position of the gill pouches is naturally connected. Contrasted with the preceding stage the interval between the row of gill pouches and the eye has become nearly doubled and in this space the tongue muscle has taken its position. The more rapid growth of the dorsal region of the head and neck, with the accompanying growth of the tongue muscle is, I believe, sufficient to account for the apparent translocation of the line of gills." We shall later see how very close to such an explanation of the gill shifting we are forced after a careful consideration of the evidence gathered from a study of sections. By the "tongue muscle" Dean refers to the large "club muscle" arrangement of Ayers which lies between the end of the velum and the first branchial gill and serves to operate the dental-plate during its vigorous feeding motions.

I fully agree with Dean that, "Both Price and von Kupffer have assumed a correspondence between branchiomery and myomery of which in this form at least I can find no evidence." Since Price's, 96, idea, which he has corrected, 04, that a large number of gills disappear during development was erroneous the considerations of Ayers and Jackson, 00, and Johnston, 05, over this point must be disregarded or modified so as to apply to the true conditions.

Development of the individual gill.—Nothing prior to this has been contributed to the development of the Myxinoid gill, the formation of its complex pouch or the cartilaginous portions accompanying it.

The entire series of gills develop alike with the exception of the simple œsophago-cutaneous duct which fails to form the pouch and has its external tube leading out in common with the gill next to it. The most anterior gill may be taken as an example since it is better developed than the following ones throughout almost the entire embryonic life. The earliest anlage of this gill consists of a slight thickening of the ectoderm and a corresponding thickening and uppushing of the endoderm, the gut endoderm at this stage being spread out over the yolk, as seen in Fig. 23. As development progresses, Fig. 24, the ectodermal pocket becomes a fold just at the base of the lateral gill lappets and the

endodermal outpushing has become more pronounced, reaching to the ectoderm. At this stage an ectodermal nerve placode appears just dorsal to the gill. Following this, the remaining seven or eight gills are still spread out on marginal lappets. The present figure was taken from the same embryo which furnished Fig. 16 of the hyomandibular cleft and Fig. 20 of the first thyroidean gill.

Fig. 25, from an embryo 15 mm. in length, shows the first gill still at the base of the lateral gill lappets with the endodermal diverticula much greater in extent and the ectodermal thickening but little changed. The extreme anterior parts of the second and third gills are indicated by the two indentations in the endoderm g2 g3. These gills are now beginning to be drawn in from the lateral lappets as the closure of the fore-gut progresses. And at this stage the hyomandibular and first thyroidean gills have already been shown in Figs. 17 and 21 respectively. The fore-gut continues to close by the drawing together of the sides of the gill lappets, and thus the gills are brought in along the sides of the neck in the manner which Dean has already described. The shifting does not begin until this process is almost completed.

Pouching of the Gills.—Before the gills begin to shift backward the endodermal diverticula are coming to form chamber-like cavities a short distance from their proximal ends. Fig. 27 shows a section through a gill at this stage the swollen cavity, gp, is seen near the pharynx, g, the connection of the gill tube with the pharynx is anterior to this section, the gills all having a trend from the gut caudally to the ectoderm as will be seen by referring to the diagram Fig. 35. This swelling of the gill tube is the earliest step in the formation of the complex gill pouch of the adult. The progressive changes in the endodermal gill chamber may be seen by studying the sections shown in Figs. 27 to 32. In Fig. 28 the wall of the chamber is beginning to be folded and in Fig. 29 the pouch is already assuming a rather complexly folded condition. In Fig. 30 the pouch is seen to communicate with the pharynx, the outer tube, *i. e.*, leading from the pouch to the ectoderm, appearing only in a more posterior section. In this section the ectoderm is seen to form a slight fold, ep, margining the end of the endodermal tube. At this time the pouch begins to grow rapidly, as will be noticed by comparing the several figures all drawn to the same scale. The gills at the stage shown in Fig. 30 have shifted far back, almost as far as in the fully developed condition. In Fig. 31 is seen a section of a somewhat further developed pouch from the mid gill region of the same embryo of which Fig. 30 represents the most anterior pouch. The pouch of Fig. 31



FIGS. 27 TO 31. The development of the pouch of the first branchial gill $\times 60$ diameters. Fig. 27. *gp*, portion of the gill tube with its walls bulged apart to form a chamber the earliest anlage of the pouch. *g*, pharynx. The gill connection with the pharynx is anterior of this section. Fig. 28. In an older embryo, the walls of the chamber beginning to fold. Fig. 29. A still older stage the pouch walls further folded. Fig. 30. The pouch increased in size and becoming more complex. Fig. 31. The cartilage arch, *Ba*, is forming about the external gill tube; *ep*, the ectodermal pocket. The pouch is larger and more complex than formerly. Fig. 32. Through a gill pouch in the mid-branchial region of a lately hatched embryo. *g*, pharynx; *et*, section of the external gill tube.

is larger and more complex, and the section shows, fortunately, a portion of the tube leading to the ectoderm. The ectoderm itself now forms a slight pocket and is reduced in thickness at this point. Around the external gill tube the cartilage arch, *ba*, is forming and it is seen to be entirely an extra-branchial arch in all cases except the oesophago-cutaneous duct. And here the cartilage extends entirely to the gut, oftentimes spreading out slightly along its walls, as Ayers and Jackson, *oo*, have also pointed out. As seen from Figs. 30 and 31 the gills in the mid region at this stage are becoming better developed than the anterior ones. Indeed in the hatched embryo the extreme anterior and posterior pouches are rarely so well formed as those nearer the middle of the line.

Finally, Fig. 32 shows a section through a gill of a lately hatched embryo. The gills now, of course, open to the exterior and are perfectly pouched. In the figure *et* is a cross section through the external gill tube which now leads from the pouch to the skin in an almost posterior direction. Thus the characteristic marsipobranch is entirely derived from the endodermal portion of the gill and reaches its adult complexity by a spreading out and subsequent folding of the walls of the originally simple gill tube. The ectodermal portion of the final gill structure is insignificant, being nothing more than a short rim about the external gill opening.

The Cartilage Arches, in their embryonic condition are, as far as I could judge from a comparison with Ayers and Jackson's description, very similar to these structures in the adult. At some stages they appear to be slightly more extensive. These differences, however, are insufficient to base conclusions upon since all parts of these animals are subject to such wide variations. The branchial skeletons of the Marsipobranchii have long been contrasted with the typical piscian condition as extra-branchial instead of intra-branchial. This distinction in *Bdellostoma* at any rate, as Ayers and Jackson maintain, is not conclusive for the reason that the cartilage of the oesophago-cutaneous duct, which is a gill tube, extends entirely into the pharyngeal wall. I think that a very plausible explanation may be offered for the condition in the other cartilage arches which will show them to be secondarily extra-branchial. It will be recalled from the preceding description that all of the gills of *Bdellostoma* are at one time in their development simple tubular structures just as the oesophago-cutaneous duct always is. Now from analogy I assume that if these gills should remain tubular until the stage in development when the cartilage forms they also would have sheaths of cartilage supporting them from their outer ends along their entire length to the pharyngeal wall just

as in the case of the œsophago-cutaneous duct. If such did occur the pouches could scarcely form. But since the pouch is well formed before the cartilage appears, and as the cartilage formations are not so extensive as to extend around these huge pouches so as to become intra-branchial structures they must remain about the external gill tube as extra-branchial. It may also be remarked, not that I think it a very strong point in the argument, that cartilage supports are entirely unessential to the efficient action of these pouched gills, while such supports are very useful in maintaining a fully spread condition of the typical fish gill. Further, when we remember that only the Marsipobranchii have pouched gills and likewise extra-branchial skeletons, while all fishes have intra-branchial arches and unpouched gills, one can scarcely doubt that some correlation exists between the processes that have given the pouches and the causes that have made the skeleton extra-branchial. Of course the various stages of the struggle, if we may so call it, between the pouches and the intra-branchial arches may well have been more complex than the present explanation supposes.

I am inclined to consider the condition of the œsophago-cutaneous duct as one of arrested development, *i. e.*, it remains in the tubular stage through which all of the other gills pass before forming their pouches. This might be called a modification but it is one which has not greatly affected the primitive condition of this structure, and argues only to a slight degree, if at all, against the above position. Further if it be claimed that the extensive cartilage support of this duct was a new or special acquisition, one might then reply that this indicates the fact that in *Bdellostoma* we have an animal on the road to possessing an intra-branchial skeleton, which is even now partially formed. The extra-branchial condition from this point of view would be, therefore, primary and not secondary. But since these animals possess a mandibular arch they must have also possessed intra-branchial gill arches because the one is to be considered only as a modification of the other. At any rate according to our present knowledge there is clearly no good reason for accepting Dohrn's view that the arches were lost on account of a change in the animal's life-habits.

From such considerations as the above one must at least admit that these animals are contrasted with fishes as having extra-branchial skeletons on rather flimsy grounds. To any one that will study these structures it will, I believe, become clear that marsipobranchs have in part, and probably once had entirely, an intra-branchial skeleton.

The Shifting of the Gills in the embryo of *Bdellostoma* was correctly described by Dean, 99, as mentioned at the beginning of the discussion

of the branchial organs. My study of the sections has enabled me to add a few further details.

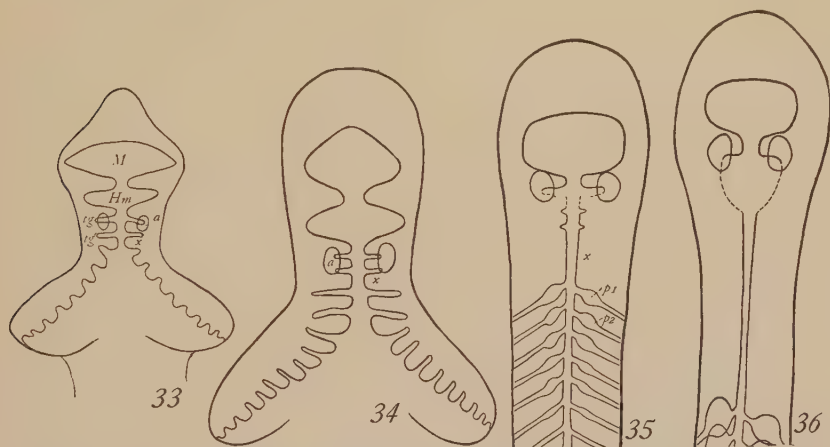
In young stages where the gills are on the laterally outspread gill lappets as illustrated in the diagrams Figs. 33 and 34 no shifting at all has taken place. Each gill follows the preceding one at approximately equal intervals. In diagram 33 M indicates the horizontal outline of the mandibular arch, Hm the same for the hyomandibular cleft, and just following this are the two pair of thyroidean gills. The last, it will be seen, are well within the head region, and are indeed at no time on the lateral gill lappets. After the thyroidean gills, tg, (which are now more advanced in development than any of the true branchial ones) the other gill folds come in regular succession. The auditory vesicles are just over the first thyroidean gills.

In more advanced embryos the gills are being folded in along the sides of the body as the gill lappets are drawn together, Fig. 34 shows the first few already brought in. The ear vesicles have increased in size and now lie over the two thyroidean gills. When the gills are all just about folded in along the neck, a region of rapid growth is formed between the second thyroidean and the first branchial, or in other words just anterior of what was the forward limit of the gill lappets, or at the point marked x in Figs. 33, 34, and 35. This rapid growth of the dorsal region of the head and neck causes the head to advance around the anterior end of the egg, leaving the gills as it were behind. Later the large club muscle of the dental-plate begins to develop in the ventral neck region between the thyroidean and the first branchial gills, thus further facilitating the lengthening of this area and helping to cause the translocation of the gills from their original place in the neck to their final position along the sides of the trunk.

The myotomes in young stages begin behind the gills, the most anterior one being posterior of the last gill, but during development many myotomes come to lie anterior of the first gill. I find no definite relation between myotome and gill arrangement at any stage. Thus one must conclude from a study of *Bdellostoma* embryos that there is no essential correspondence between myomery and branchiomery.

The exact growth point in the shifting process can be located since the second thyroidean gill undergoes its entire degeneration without changing its relative position, while the next gill which followed it so closely comes finally to lie a long way posterior to its place of origin. Since also the second thyroidean is the most posterior cleft which does not arise on the lateral gill lappet and the first branchial is the first one of the lappet series the idea at once suggests itself that the lappet area may be as it

were loosely associated with the dorsal muscular tissue, consequently in the rapid growth of this dorsal tissue the lappet structure with the gills is left behind. The complex changes connected with the enormous growth of the dorsal neck tissue, the development of the large club-muscle, and the connection of the gill lappet tissue with the dorsal musculature are the principal causes operating to produce the translocation of the gills in the embryo.



FIGS. 33 to 36. Diagrams illustrating the change in position relations during the development of the gills. Fig. 33. The condition in young embryos when all of the branchial gills are out on the gill lappets. *a*, ear vesicle; *M*, outline of the mandibular diverticulum; *Hm*, the hyomandibular; *tg*, *tg'*, first and second post-hyomandibular gills; *x*, point where rapid growth takes place causing the head to grow forward leaving the gill area behind. Fig. 34. The first few gills are drawn in from the lateral lappets. *x*, the rapid growth point; *a*, ear vesicle increased in size. Fig. 35. An embryo in which the gills have partially shifted. The two post-hyomandibular ones are in their original position and have decreased in extent. *p1*, *p2*, the beginning of the pouches in the branchial gills. Fig. 36. The gills shifted far back. The ear vesicles are partially anterior to the hyomandibular. The indefinite posterior border of the hyomandibular cleft is dotted.

Fig. 35 shows the gills partially shifted back: the pouches, *p*, are forming near the communications of the gill tubes with the pharynx, the thyroidean gills have greatly degenerated and the auditory vesicles are seen to have moved forward in their relation to the mandibular arch. This forward change at such a period is probably due to a shortening or forward condensation of the embryonic brain. Fig. 36 indicates the further change in the gill position. In Figs. 35 and 36 the posterior limit of the hyomandibular clefts are dotted to indicate that they fade out over the velum in a very indefinite manner as above referred

to. The club muscle develops entirely between the dotted lines and the first gill, being thus confined to this area of rapid growth.

As stated in a previous paper on the development of the thyroid gland in *Bdellostoma*, this gland and the other organs of the gill region develop entirely *in situ* not shifting or moving during their development. For the gill shifting process is not an actual moving of the gills as will be clearly understood from the explanation which has just been given.

CONCLUSIONS.

My studies up to this point have not yet made it possible for me to satisfactorily interpret Kupffer's, oo, "primary mouth" in *Bdellostoma*, a structure he describes from very young embryos. But I must admit that I am extremely skeptical regarding this earliest mouth. At times I am led to believe that this was really the nose which in the case of very early embryos, as I find, leads directly into the throat. The most convincing thing in Kupffer's argument is the following paragraph regarding the "secondäre Rachenhaut," a structure which undoubtedly exists.

"Bei dieser Sachlage warfen sich verschiedene Fragen auf. Zunächst die, ob die Epithelplatten, durch welche beide Canäle äusserlich geschlossen werden, primäre Bildungen sind, oder ob es sich hier um einen secundären Verschluss vorher klaffender Oeffnungen handelt. Im ersteren Falle wäre die den Munddarm verschliessende Platte als Rachenhaut aufzufassen und am Hypophysencanal fände sich eine analoge Vorrichtung. Dann aber wäre das Epithel beider Canäle ein endodermales und es ergäbe sich hieraus der paradoxe Schluss, dass das Epithel der Nase nur an das Emdoderm Anschluss hätte. Viel einfacher würde sich aber die Deutung vorliegender Verhältnisse gestalten bei der Annahme, dass beide Canäle erst secundär sich geschlossen haben."

Dean, 99, also states on page 270, referring to an old embryo that, "It seems clear to me, however, that the mouth and nasal openings in the present instance are certainly to be looked upon as of secondary acquisition." I must express my conviction as to the truth of the two above statements, and must regret that I have not been able in the young embryos to find the satisfactory stages showing the primary mouth opening and its subsequent closure by the "secondäre Rachenhaut."

There is much in these embryos to suggest the correctness of Dohrn's idea that the nasal tube, naso-hypophyseal canal, in myxinoids is the homologue of the ancient vertebrate mouth.

Regarding the relationships of the myxinoids I should say that it is

evident from the foregoing facts that they are gnathostomatous vertebrates, and therefore the name cyclostomes should no longer be used in reference to them. The term Marsipobranchii proposed by Bonaparte in 1846 and adopted by Huxley, Parker, Beard, and others may very fitly be used to designate this group. This suggestion has been so frequently made in the literature of the subject that it sounds like a very old cry, nevertheless it is one that should be heeded as it will eliminate from our classification a term whose meaning is undoubtedly misleading, as well as one which will hinder the recognition of these animals as true jaw-bearing vertebrates.

As to the position of the Marsipobranchii in the gnathostome series I have no definite opinion. These animals are, however, undoubtedly primitive as is indicated by the development of their mandibular arch and the presence of the additional anterior gill clefts along with other anatomical features such as a simple ear, straight digestive tract, etc. They are in other respects decidedly specialized, as is indicated by the development of the dental-plate with its musculature, which is in reality the modified lower jaw apparatus. The peculiar pouched gills must also be regarded as specialized. The only point that I know of in the *Bdellostoma* embryo to suggest degeneracy is the fact that a lens-like thickening of the ectoderm forms over the optic cup and later disappears. I should rather, however, interpret this as a case of arrested development since the adult eye is embryonic in its appearance, as was shown by Allen, 05. Lewis, 04, has also shown that when the optic cup of the amphibian eye is removed, or does not continue its development, the lens will degenerate in the embryo. All of those features in *Bdellostoma* that have been accounted for as due to its parasitic or degenerate condition must be, in my opinion, explained on some other grounds.

SUMMARY.

1. In the development of the mandibular cleft of *Bdellostoma* the lateral diverticula at first turn dorsally, fusing with dorsal ectodermal plates in the same manner that all gills do. The diverticula later extend horizontally, and finally, bending ventrally, come to resemble the mouth arch of most other vertebrates. The fusion of the ectoderm and endoderm continues through all the stages of development.

These conditions suggest forcibly Dohrn's idea of the origin of the vertebrate mouth from a pair of gill slits.

2. The so-called "tongue" of myxinoids is really the homologue of the gnathostome lower jaw.

a. Its earliest anlage comprises the entire mass of tissue ventral of the mandibular cleft, and from similar tissue in all other gnathostomes the elements of the lower jaw are differentiated during development.

b. The "tongue" should better be called dental-plate since its muscles are not comparable with the tongue muscles of other vertebrates, and since it is innervated by the true lower jaw nerves, r. mandibularis of the trigeminus, as shown by many workers.

c. The dental-plate originates too far forward in the embryo to be a tongue, it is not associated with the hyobranchial apparatus, and its bilateral manner of development with its paired cartilage is jaw-like.

d. The development of the horny teeth on its surface and many other features of its later growth are jaw-like.

e. The ventral wall of the dental-plate of old embryos forms also the ventral wall of the head; and the later development of this organ makes it clear that the longitudinal mouth of myxinoids is only a slightly altered transverse mouth.

3. The hyomandibular cleft is enormously developed in young embryos, having well-formed ectodermal pockets in direct contact with the endodermal diverticula. A retrogressive development soon commences and the gut diverticula gradually decrease in size and finally disappear. Similarly all traces of the associated ectodermal pockets are finally lost.

4. Two post hyomandibular gills occur in the early embryos and are for some time in advance of the true branchial gills in their state of development. They then begin to degenerate, and rapidly disappear. One of these gill pairs may correspond to the thyroidean gill of Dohrn. They are interesting since the maximum number of gills in chordates appears thus increased from fifteen to seventeen.

5. The large and complex gill pouches of the adult are derived from the simple endodermal gill tube of the embryo by a spreading apart and subsequent folding of its walls in the region near its communication with the pharynx.

6. The cartilaginous branchial arches of the embryo are scarcely different from those of the adult. There are reasons for believing that the branchial skeleton of the Marsipobranchii is not essentially different from that of fishes: the extra-branchial condition is not entire and is probably secondary.

7. A great change in relative position appears during the development of the gills. The gills originate in the posterior head or neck-region of the embryo and come finally to lie far back along the sides of the trunk. A region of rapid growth can be located between the second thyroidean

and first branchial gills. The complex changes connected with the enormous growth of this neck tissue, together with the development of the large "club muscle," and the loose connection of the gill lappet tissue with the dorsal body musculature are the main causes operating to produce the translocation of the gills.

8. From this study of the head organs in embryos of *Bdellostoma* I am led to believe this animal in many respects primitive, while in others it is no doubt specialized. There are no reasons for believing it parasitically degenerate.

Since it is a true jaw bearing vertebrate the term cyclostome should no longer refer to it, the less objectional one marsipobranch should be used entirely.

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